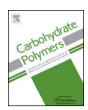
ELSEVIER

Contents lists available at ScienceDirect

# Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol



# Alginate mediate for synthesis controllable sized AgNPs



M.K. Zahran<sup>a</sup>, Hanan B. Ahmed<sup>a,\*</sup>, M.H. El-Rafie<sup>b</sup>

- <sup>a</sup> Chemistry Department, Faculty of Science, Helwan University, Ain-Helwan, Cairo 11795, Egypt
- <sup>b</sup> Textile Research Division, National Research Centre, Dokki, Cairo 12311, Egypt

#### ARTICLE INFO

Article history: Received 17 February 2014 Received in revised form 8 March 2014 Accepted 15 March 2014 Available online 18 April 2014

Keywords: AgNPs-alginate composite Reducing sugars TEM

#### ABSTRACT

A new method to prepare silver nanoparticles was reported. Alginate colloidal solution containing chemically synthesized silver nanoparticles (AgNPs) was investigated regarding the nanoparticles stabilization and possibilities for production of alginate based nanoparticles. The formation of AgNPs has been confirmed by UV-visible spectroscopy and monitoring of reducing sugars in the reaction was carried out. The morphology of synthesized silver nanoparticles was characterized by transmission electron microscopy (TEM). The results showed that the morphology of Ag nanoparticles is spherical and the main size is about 1–4 nm

© 2014 Elsevier Ltd. All rights reserved.

# 1. Introduction

Alginate, a natural-occurring polyelectrolytic polysaccharide found in all species of brown algae and some species of bacteria, is a linear polymer composed of  $\alpha$ -L-guluronate (G) and  $\beta$ -D-mannuronate (M) units in varying proportions and sequential arrangements, and is biocompatible and biodegradable in tissue (Bouhadir et al., 2001; Gombotz & Wee, 1998; Robitaille et al., 1999). Alginates have also been widely studied for their ability to form gels in the presence of divalent cations (Finotelli, Morales, Rocha-Leao, Baggio-Saitovitch, & Rossi, 2004; Nesterova, Walton, & Webb, 2000; Sreeram, Shrivastava, & Nair, 2004). Also, sodium alginate is found to be well dissolved in water due to negatively charged carbonyl groups.

Metal alginate gels are ionotropic in nature, differing from the classical type of gels in which long-chain molecules are held together by simple van der Waals forces, and the macromolecular chains can chelate some of polyvalent metal ions (Khairou, Al-Gethami, & Hassan, 2002). The design and construction of metal-containing nanoscale arrays is a challenge since such structures are expected to be indispensable in the emerging technologies of this century.

Silver nanoparticles (AgNPs) are the focus of extensive research over several decades due to their many possible applications, such as antimicrobial agents, catalysts and optical sensors (Abdel-Mohsen, Aly, & Hrdina, 2012; Abdel-Mohsen et al., 2014; Huang, Liao, Ling, & Wen, 2009; Pal, Shah, & Devi, 2009; Rastogi & Arunachalam, 2011; Saha, Pal, Kundu, Basu, & Pal, 2010; Yoksan & Chirachanchai, 2009). Wound infection contributes to the formation of a nonhealing wound. Bacterial load on the surface of a wound amplifies and/or perpetuates a pro-inflammatory environment. Therefore, antimicrobial agents, such as silver-based formulations, are often used for wound healing. Silver-coated dressings are commonly used because they are effective in killing a broad range of bacteria. However, the utility of these nanoparticles largely depends on the carrier that is used for prevention of particle aggregation.

The preparation of a uniform and stable colloidal dispersion of AgNPs, free from agglomeration and precipitation, is a core technique. On the other hand, stable dispersions of AgNPs are generally short-lived in an aqueous media because they tend to agglomerate. For the preparation of metal particles, metal ions have often been reduced in protective colloids. Agglomeration is traditionally overcome through spontaneous adsorption on the particle surface of polymeric stabilizers. As stabilizers and protective media for colloids, water-soluble polymers have been used (Aslam, Fu, Su, Vijayamohanan, & Dravid, 2004; Brugger, Guendet, & Graetzel, 1981; Dunworth & Nord, 1954; Esumi, Sato, Torigoe, & Meguro, 1992; Esumi, Wakabayashi, & Torigoe, 1996; Hebeish, El-Rafie, Abdel-Mohdy, Abdel-Halim, & Emam, 2010; Hirai, Nakao, & Toshima, 1979; Hirai, 1979; Huang et al., 1996; Kattumuri et al., 2007; Kim, Park, Lee, Jeong, & Jon, 2007; Kiwi & Graetzel, 1979; Kuo, Chen, & Jao, 2005; Luo, Zhang, Zeng, Zeng, & Wang, 2005; Sun & Xia, 2002; Sun, Dong, & Wang, 2004; Sun, Dong, & Wang, 2005; Toshima, Harada, Yamazaki, & Asakura, 1992; Yamamoto

<sup>\*</sup> Corresponding author. Tel.: +20 1097411189; fax: +20 1097411189. *E-mail addresses:* hananbasiony@yahoo.com, hananbasiony@gmail.com (H.B. Ahmed).

& Nakamoto, 2003; Yonezawa & Toshima, 1995). Water-insoluble polymeric materials also have been recently used (Hossam et al., 2013).

Polymers, in solutions as well as in the hydrogel form, have shown to be quite efficient as capping agents and/or nanoreactor matrixes for the synthesis of metal nanoparticles (Leung, Wong, & Xie, 2010; Mohan, Lee, Premkumar, & Geckeler, 2007). Alginate is particularly attractive for biomedical applications, since it is biocompatible, biodegradable, hydrophilic, and easily processed into different hydrogel shapes (Marijnissen et al., 2002).

Recently, AgNPs were synthesized in alginate solutions using gamma irradiation (Liu, Chen, Zhong, & Wu, 2009), and on the surfaces of alginate microbeads using photochemical reduction (Saha et al., 2009, 2010). Alginate microbeads incorporated with AgNPs can be used as biocompatible carriers and/or as efficient donors of nanoparticles for active components in all applications in which they are utilized (e.g. medicine, pharmacy, catalysis, optics) (Travan et al., 2005). Alginate–chitosan microbeads incorporated with AgNPs were produced by a rather complex procedure involving chemical synthesis of AgNPs in chitosan solution followed by addition of D-glucono- $\alpha$ -lactone, CaCO<sub>3</sub> and Na-alginate and electrostatic extrusion of the obtained mixture (Travan et al., 2005).

Herein, we reported the development of a novel, facile and 'green' route for synthesizing AgNPs using alginate as both reducing and stabilizing agent in aqueous alkaline medium by heating. The synthesized alginate capped AgNPs were characterized and found to exhibit antibacterial properties. Several studies, in this context, have demonstrated successful incorporation of AgNPs in food packaging films (Tankhiwale & Bajpai, 2009) and coatings on medical devices (Roe, Karandikar, Bonn, Gibbins, & Roullet, 2008) as antimicrobial agent. Thus, our next objective was to apply films of alginate capped AgNPs to be coated on cotton fabrics in order to produce new fabrics useful for medical applications.

This study is concerned with discovering novel, easily applicable and green approach for synthesizing AgNPs with different shapes and sizes using alkali hydrolyzed sodium alginate which is naturally occurring, and environmentally friendly polymer, to play the dual role, as a reducer of AgNO<sub>3</sub> and stabilizing agent for the produced AgNPs. The concentration of reducing sugars in the reaction mixture was measured using DNS (dinitrosalicylic acid) reagent in order to follow up the redox reaction between alkali hydrolyzed alginate and silver nitrate. UV–visible spectroscopic data was systematically studied for indicating the presence AgNPs under different experimental conditions. Transmission electron microscope (TEM) images were presented to confirm the presence of silver nanoparticles in reaction medium and to study the particle size and size distribution of the prepared nanosilver under the different experimental conditions.

#### 2. Experimental

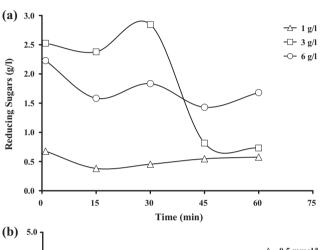
# 2.1. Chemicals

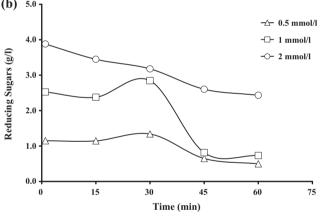
Silver nitrate (99.5%), sodium alginate (91–106%) supplied from (Loba Chemie, Mumbai, India), sodium hydroxide, 3,5-dinitro salicylic acid (DNS), sodium sulphite, potassium sodium tartarate, glucose, phenol, and sodium carbonate monohydrate were all used without any further purification.

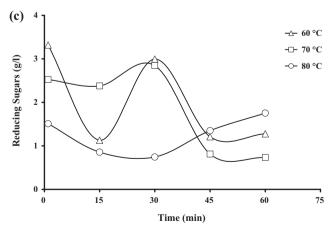
# 2.2. Method of preparing AgNPs-alginate composite

Definite weights of sodium alginate  $(1-6\,g/l)$  were hydrolyzed by sodium hydroxide solution using magnetic stirrer to prepare different solutions of alkali hydrolyzed alginate with pH value  $\approx 12$  in order to serve the dual role as a reductant of silver nitrate and

stabilizer for the prepared nanosilver particles. After a complete dissolution, the temperature of the reaction medium was raised to the desired degree ( $60-80\,^{\circ}$ C). In this moment, certain amount of silver nitrate solution ( $0.1\,\mathrm{mole/l}$ ) was added dropwise (keeping in mind that the total volume of the reactants is  $100\,\mathrm{ml}$ ). The reaction was kept under continuous stirring for different durations ( $1\,\mathrm{min}$  up to  $60\,\mathrm{min}$ ). After addition of silver nitrate, the reaction medium acquires a yellowish color turned to brownish color by time, indicating the formation of silver nanoparticles. The progression of the reaction was controlled by UV–visible absorption; aliquots from the reaction bulk were withdrawn at a given time intervals and has been evaluated.







**Fig. 1.** (a) Reducing sugar content as a function of alginate concentration using 1 mmole/l AgNO $_3$  at 70 °C. (b) Reducing sugar content as a function of silver nitrate concentration, using 3 g/l alginate at 70 °C. (c) Reducing sugar content as a function of temperature, using 3 g/l alginate and 1 mmole/l AgNO $_3$ .

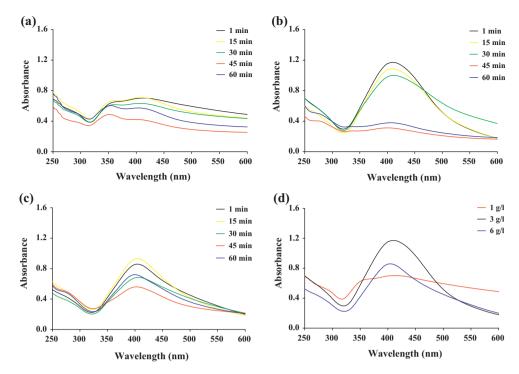


Fig. 2. UV-vis spectra of silver nanoparticles solutions, using 1 mmole/l AgNO<sub>3</sub> at 70 °C. (a) 1 g/l alginate, (b) 3 g/l alginate, (c) 6 g/l alginate and (d) UV-vis spectra of silver nanoparticles solutions by using different concentrations of alginate, using 1 mmole/l AgNO<sub>3</sub>, at 70 °C for 1 min.

# 3. Chemical analyses

# 3.1. Determination of the concentration of residual reducing sugars using DNS reagent

The dinitrosalicylic acid reagent (DNS) was used for determining the concentration of residual reducing sugars in the reaction medium under different experimental conditions. This method tests for the presence of the free carbonyl group of the reducing sugars remained after the redox reaction between silver nitrate and alkali hydrolyzed alginate. This involves the oxidation of the aldehydic and/or ketonic groups present in the sugars to the carboxylic group. However, 3,5-dinitrosalicylic acid is reduced to 3,5-diaminosalicylic acid under alkaline conditions.

The reagent is composed of dinitrosalicylic acid, Rochelle salt (sodium potassium tartarate), phenol, sodium bisulfite, and sodium hydroxide. According to Sumner (1921), Rochelle salt is added to prevent the reagent from dissolving oxygen, phenol is used to increase the amount of color produced, and bisulfite to stabilize the color obtained in the presence of phenol. Also the alkali is required for catalyzing the reducing action of sugar on dinitrosalicylic acid.

The test was carried out with 3 ml of DNS reagent which is added to 3 ml of different colloidal silver nanoparticles solutions under different conditions in capped test tubes. The mixture was heated at 90 °C for 15 min to develop the reddish brown color. A definite volume (1 ml) of Rochelle salt is added to stabilize the color. After cooling at room temperature in a cold water bath, the absorbance was recorded spectrophotometerically at 575 nm (Sumner, 1921).

## 3.2. UV-visible spectroscopy

Silver nanoparticles solutions exhibit an intense absorption peak due to the surface plasmon resonance (SPR). Thus the UV-visible absorption spectra were used to prove the formation of AgNPs colloidal solutions. The UV-visible absorption spectra of AgNPs colloidal solutions were measured using a multi

channel spectrophotometer (T80 UV/VIS, d = 10 mm, PG Instruments Ltd, Japan).

# 3.3. Transmission electron microscopy (TEM)

The morphology and the distribution of AgNPs were characterized by means of a JEOL-JEM-1200 Transmission Electron Microscopy, a two drops of the silver nanoparticles colloidal solutions were placed on a 400 mesh copper grid coated by carbon film and then the grids was conducted the microscope. The samples were prepared by placing a drop of the colloidal solution on a 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature.

# 3.4. Particles size distribution

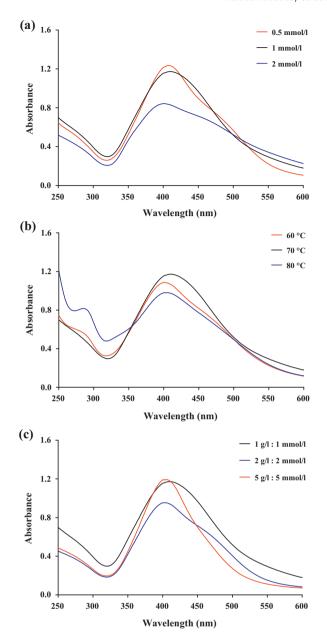
The diameter and distribution of silver nanoparticles were calculated by 4 pi analysis software using TEM photos.

# 4. Results and discussion

AgNPs have been synthesized in a 'green' method using natural biopolymer alginate by simply heating an aqueous alkaline mixture of sodium alginate and AgNO<sub>3</sub>. Here, alginate plays a dual role of both reducing agent of nanosilver generator and stabilizer for the produced nanoparticles. This method was found to produce AgNPs reproducibly.

# 4.1. Determination of residual reducing sugars using dinitrosalicylic acid reagent (Miller, 1959)

Alkali hydrolysis of sodium alginate was carried out in this study to improve its water solubility, and provide more fragments with higher reducibility, dispersibility and stability which are required for the production of well dispersed stable colloidal silver nanoparticles solutions, which is suitable for different industrial and medical applications.

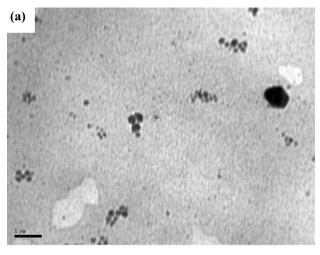


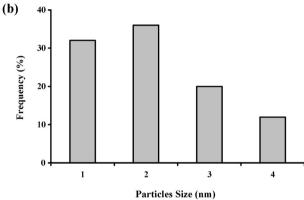
**Fig. 3.** (a) UV–vis spectra for AgNPs colloidal solutions by using different concentrations of AgNO $_3$  (0.5–2 mmole/I), with 3 g/l alginate, at 70 °C for 1 min. (b) UV–vis spectra for AgNPs colloidal solutions at different temperatures, using 3 g/L alginate and 1 mmole/I AgNO $_3$  for 1 min. (c) UV–vis spectra for AgNPs colloidal solutions at different ratios between alginate and AgNO $_3$ ; the experiments are carried out at 70 °C for 1 min.

Sodium alginate is a linear copolymer of 1,4-linked  $\beta$ -D-mannuronate (M) and  $\alpha$ -D-guluronate (G) residues. With alkali hydrolysis of alginate, the polymeric chains are supposed to degrade giving more fragments (contains greater numbers of hydroxyl and negatively charged carbonyl groups) with higher reducibility, which are well dissolved in water.

The method of measuring residual reducing sugars content depends on the presence of free carbonyl groups of the reducing sugars produced from alginate fragmentation. During the redox reaction between sugars (alginate fragments) and silver nitrate, the sugars are turned to the oxidized form with more aldehydic and/or ketonic groups.

Thus, this method involves the oxidation of the aldehydic and/or ketonic groups of sugars presenting in the reaction medium to the corresponding carboxylic acid, and simultaneously,





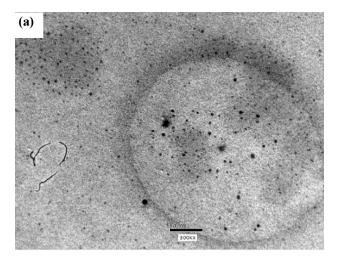
**Fig. 4.** (a) TEM micrograph of silver nanoparticles prepared with 1 mmole/l AgNO<sub>3</sub> and 3 g/l alginate at 70 °C for 1 min. (b) Size distribution and size average for silver nanoparticles in the viewed TEM image.

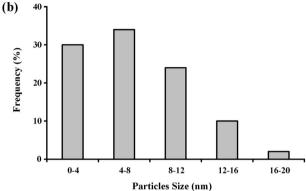
3,5-dinitrosalicylic acid is reduced to 3,5-diaminosalicylic acid. As this method depends upon that all higher oligosaccharides starting with maltose would produce equivalent amounts in color with DNS reagent, i.e., increasing of color intensity means higher concentrations of alginate fragments. Thus it could be suggested that, increasing the concentration of reducing sugars confirms the redox reaction between sodium alginate and silver ions and so means signifying the nanosilver nucleation and clusters building.

# 4.1.1. Effect of sodium alginate concentration

From data shown in Fig. 1a, it could be observed that, the highest concentration of residual reducing sugars is obtained by using 3 g/l alginate, and increasing the concentration of alginate to 6 g/l is accompanied by decrement in the concentration of the produced reducing sugars. Also an enhancement of reduction process from the first minute could be observed, so this means that the alginate fragmentation is proceeded giving the highest amounts of reducing sugars from the first minute, which in turn required for silver nanoparticles preparation. For 1 g/l alginate, the concentration of reducing sugars is nearly stable with time, however, for higher concentrations of alginate 3 g/l and 6 g/l, the greatest concentration of reducing sugars is obtained from the first minute and nearly the concentration is stable till 30 min, then starts to decrease with time, which could be attributed to polymer destruction (Nadezhda, Natalya, Lydmila, & Vasilyi, 2012).

Also the alkali hydrolyzed alginate solution without silver nitrate was monitored with time and the concentration of reducing sugars was not changed significantly  $(0.149 \pm 0.009 \text{ g/l})$ , confirming





**Fig. 5.** (a) TEM micrograph of silver nanoparticles prepared with 1 mmole/l  $AgNO_3$  and 3 g/l alginate at 70 °C for 60 min. (b) Size distribution and size average for silver nanoparticles in the viewed TEM image.

that reducing sugars are resulted as products of the redox reaction which takes place between alginate fragments and silver nitrate.

### 4.1.2. Effect of silver nitrate concentration and temperature

From Fig. 1b, it could be suggested that, when the concentration of silver nitrate was increased, the concentration of residual reducing sugars was raised, as by increasing the concentration of silver nitrate, the rate of redox reaction is increased, resulting in higher concentration of reducing sugars. However, the maximum concentration of reducing sugars is obtained from the first minute, and by prolonging time, the concentration of reducing sugars starts to decrease, which could be attributed to polymer destruction (Nadezhda et al., 2012).

From Fig. 1c, from the first minute, the maximum concentration of reducing sugars was detected for both 60 and 70 °C. So, it could be decided that, higher temperature plays an important role in accelerating the redox reaction between silver ions and alginate fragments. However, by raising the reaction temperature, the concentration of reducing sugars starts to decrease, which may be also attributed to polymer destruction (Nadezhda et al., 2012).

# 4.2. UV-vis spectra

UV-visible spectrum is recorded in the absorbance mode in the range 250–600 nm. Regardless of the parameters of synthesis (i.e. concentration of sodium alginate, AgNO<sub>3</sub> concentration in the initial alginate solution, temperature and time), UV-vis analysis has shown that Ag/alginate colloid solutions exhibited surface plasmon absorption band peaking in the wavelength range 405–440 nm,

corresponding to particles whose radii are smaller than 30 nm (Mulvaney, 1996). These results correspond to a plasmon resonance effect originating from the quantum size of AgNPs (Mulvaney, 1996) and thus confirmed the presence of Ag nanoparticles in Ag/alginate colloid solutions.

UV-vis spectra were used also to determine the effects of all of the parameters of synthesis on the amount and relative size of silver nanoparticles formed in different Ag/alginate colloid solutions. It must be noted that, all samples under different experimental conditions are diluted before measuring process till the concentration of the produced nanosilver reaches 10 ppm.

# 4.2.1. Effect of alkali hydrolyzed alginate concentration

In order to optimize the synthesis of AgNPs, the concentration of alginate was varied (1, 3 and 6 g/l) while keeping AgNO<sub>3</sub> concentration and reaction temperature constant at (1 mmole/l) and 70  $^{\circ}$ C, respectively.

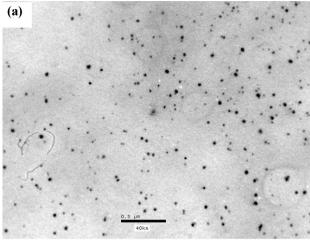
After the first minute of reaction, the color of the reaction mixture changed from yellow to light brown to dark brown with increasing alginate concentration, indicating the formation of AgNPs. The data shown in Fig. 2a-c suggest that, the absence of an SPR band in UV-vis spectrum at 1 g/l indicated that no AgNPs were formed, as the concentration was increased to 3 g/l or above, a well defined plasmon band appeared at 405-410 nm from the first minute of the reaction which is characteristic for AgNPs, and further increase in the reaction duration leads to decreasing in absorbance. As is evident from Fig. 2d, the intensity of the peak increased till the concentration of alginate reaches 3 g/l, indicating complete reduction of silver ions at this concentration of alginate. By increasing the concentration of alkali hydrolyzed alginate up to 6 g/l the maximum absorbance value is decreased which may suppose that the produced nanoparticles may be aggregated.

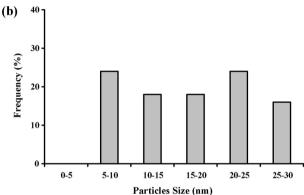
## 4.2.2. Effect of silver nitrate concentration and temperature

Fig. 3a shows the dependence of the maximum absorbance in Ag/alginate colloid solutions on the AgNO<sub>3</sub> concentration, since, higher concentrations of Ag<sup>+</sup> ions in the initial alginate solution resulted in higher concentrations of nanoparticles, as the maximum absorbance value is well observed from the first minute with 1 mmole/l AgNO<sub>3</sub>. However, it could be observed that, by increasing the concentration of silver nitrate up to 2 mmole/l, the maximum absorbance value is decreased. Besides, regardless to the silver nitrate concentration, the maximum absorbance value is decreased by time, which may suggest that, by increasing silver nitrate concentration and also by time, the produced nanoparticles are supposed to be aggregated and/or agglomerated.

Fig. 3b shows the UV-vis absorption spectra of AgNPs prepared at different temperatures (60, 70, and 80 °C) using initial pH 12 with time. It is clear from the data that (a) the temperature plays the important role to reduction reaction, (b) when the reaction temperature was 60 °C the color of the solution was light yellow, and the plasmon band was appeared around 410 nm which indicate starting of converting Ag<sup>+</sup> to Ag<sup>0</sup>, (c) raising the reaction temperature up to 70 °C is accompanied by the formation of deep yellow color and the absorption band at 410 nm becomes stronger and narrower which means higher conversion of Ag<sup>+</sup> to Ag<sup>0</sup> with smaller nanoparticles size and (d) further increases in the reaction temperature to 80 °C leads to decrease in the maximum absorbance value and appearance of a peak around 300 nm which may suggest that, the produced nanoparticles at temperature higher than 70 °C may tend to collapse and then aggregated to regenerate silver ions in the reaction medium (Hebeish et al., 2010).

Regardless to the reaction temperature, prolonging the reaction duration from 1 to 60 min for all prepared samples leads to a





**Fig. 6.** (a) TEM micrograph of silver nanoparticles prepared with 2 mmole/l AgNO<sub>3</sub> and 3 g/l alginate at 70 °C for 1 min. (b) Size distribution and size average for silver nanoparticles in the viewed TEM image.

decrease in the maximum absorbance value which is also explained due to aggregation of the produced nanoparticles.

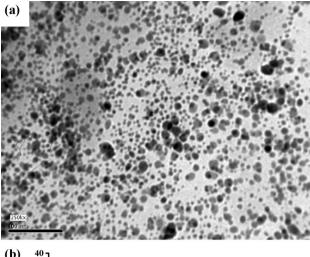
# 4.2.3. Effect of different ratios between alginate and AgNO<sub>3</sub>

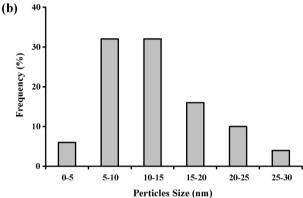
In order to produce maximum concentration of nanosilver colloidal solution with the highest stability, certain ratio of silver nitrate to alkali hydrolyzed alginate must be prepared. Hence, preparation of silver nanoparticles was carried out using higher concentrations of both silver nitrate and alginate. Increasing the concentration of silver nitrate and alginate was done while the ratio of silver nitrate to alkali hydrolyzed alginate was maintained.

Fig. 3c shows the UV–vis spectra of the silver colloidal solutions obtained using different ratios of alkali hydrolyzed alginate and silver nitrate. It could be significantly observed that: by increasing the ratio from 1:1 (for preparing108 ppm AgNPs) up to 5:5 (for preparing 540 ppm AgNPs), the maximum absorption peak at 410 nm becomes stronger and narrower which indicates that silver nanoparticles are formed in higher amounts with small size. Thus, it could be suggested that, the optimum conditions for preparing 1080 ppm AgNPs using alkali hydrolyzed alginate is, 30 g/l alginate at pH 12 and 10 mmole/l AgNO<sub>3</sub>, at 70 °C for 1 min.

# 4.3. Transmission electron microscopy (TEM) and particles size

The transmission electron microscope has provided further information about the morphology and size distribution of the synthesized silver nanoparticles. Representative TEM images and histograms of particle size distribution recorded from AgNPs





**Fig. 7.** (a) TEM micrograph of silver nanoparticles prepared with  $5 \text{ mmole/l AgNO}_3$  and 15 g/l alginate at  $70 \,^{\circ}\text{C}$  for 1 min. (b) Size distribution and size average for silver nanoparticles in the viewed TEM image.

produced by the modified polysaccharide as a novel method was presented in Figs. 4–7.

To confirm the results brought about by UV-vis absorption spectroscopy, the particle size was established by recording TEM of AgNPs prepared by using alkali hydrolyzed alginate. Figs. 4a, 5a, 6a and 7a illustrate the TEM micrographs for the prepared AgNPs. Figs. 4b, 5b, 6b and 7b show the histograms of particle size and particle size distribution for AgNPs prepared by different concentrations of alkali hydrolyzed alginate.

A close examination of the aforementioned figures indicates that 3 g/l of alkali hydrolyzed alginate is accompanied by significant improvement in stabilization of the formed AgNPs with smaller size. Size distribution was observed in the range 1–4 nm with major size of the particles 2 nm. Further increase in the polysaccharide concentration to 6 g/l leads enlarged size for the produced AgNPs (data not shown).

TEM images of AgNPs at an earlier stage, i.e., 1 min, exhibit smaller sized spherical particles (Fig. 4b). The histogram (Fig. 5b) clearly illustrate that prolonging time leads to that most of the produced nanoparticles exhibits enlarged particle size as particles size distribution 0–20 was recorded with major size 0–8 (65%).

Fig. 6a and b also show the effect of using different concentrations of silver nitrate on the prepared AgNPs. Results of the foregoing section made it possible to prepare silver nanoparticles solutions with a concentration (100 ppm). This concentration is rather low for industrial applications. Interest in preparation of AgNPs solutions, which acquire higher concentrations of the nano-sized silver particles are, therefore, stimulated. Thus a study was undertaken where silver nitrate (AgNO<sub>3</sub>) was incorporated

at different concentrations in the reaction medium. From the data shown in these figures it could be summarized that, increasing the concentration of silver nitrate leads to produce AgNPs much greater in size as most of the synthesized nanoparticles prepared by 2 mmole/I AgNO<sub>3</sub> have average size distribution of 5–30 nm. So it could be suggested that increasing silver nitrate concentration results in producing enlarged nanoparticles, and these findings is in agreement with that of UV–vis spectroscopy.

Fig. 7a and b shows TEM micrographs and histogram of 500 ppm AgNPs prepared for industrial applications and it could be illustrated that, the duplication of the concentration of both alkali treated alginate and silver nitrate results in the production of greater amounts of AgNPs with insignificant increase in particle size. The distribution of particles size was 0–30 nm with mainly average size of 5–15 nm which presented 63%. This confirms the suggestion of UV–vis spectroscopic data which clarify that, alkali hydrolyzed alginate could be used to prepare stable and well dispersed 500 ppm AgNPs and no aggregation is noticed. Thus, it could be concluded that alkali hydrolyzed alginate is an excellent reducer of AgNO<sub>3</sub> and a powerful stabilizer of a colloidal and a well dispersed AgNPs.

#### 5. Conclusion

A simple, safe, one-step, cost effective and eco-friendly approach is developed for the preparation of silver nanoparticles in aqueous alkali hydrolyzed sodium alginate solution from the first minute. Alkali hydrolyzed alginate played the dual role of a reducer of nanosilver generator and stabilizing agent for the synthesized silver nanoparticles. The structural analyses (UV-visible spectra and TEM images) of the AgNPs synthesized by this method and the results showed that the novel method generated the smallest AgNPs (average size of 1–4 nm) with the narrowest size distribution. The AgNPs synthesized by this method were highly stable and did not show any aggregation. Application of silver nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial agents.

#### References

- Abdel-Mohsen, A. M., Aly, A. S., & Hrdina, R. (2012). A novel method for the preparation of silver/chitosan-O-methoxy polyethylene glycol core shell nanoparticles. Journal of Polymers and Environment, 20, 459–468.
- Abdel-Mohsen, M., Rasha, M. A., Moustafa, M. G., Fouda, L., Vojtova, L., Uhrova, A. F., et al. (2014). Preparation, characterization and cytotoxicity of schizophyllan/silver nanoparticle composite. Carbohydrate Polymers, 102. 238–245.
- Aslam, M., Fu, L., Su, M., Vijayamohanan, K., & Dravid, V. P. (2004). Novel one-step synthesis of amine-stabilized aqueous colloidal gold nanoparticles. *Journal of Materials Chemistry*, 14, 1795–1797.
- Bouhadir, K. H., Lee, K. Y., Alsberg, E., Damm, K. L., Anderson, K. W., & Mooney, D. J. (2001). Degradation of partially oxidized alginate and its potential application for tissue engineering. *Biotechnology Progress*, 17, 945–950.
- Brugger, P. A., Guendet, P., & Graetzel, M. (1981). Ultrafine and specific catalysts affording efficient hydrogen evolution from water under visible light illumination. *Journal of the American Chemical Society*, 103, 2923–2927.
- Dunworth, W. P., & Nord, F. F. (1954). Noble metal synthetic polymer catalysts and studies on the mechanism of their action. *Advances in Catalysis*, 6, 125–141.
- Esumi, K., Sato, N., Torigoe, K., & Meguro, K. (1992). Size control of gold particles using surfactants. *Journal of Colloid and Interface Science*, 149, 295–298.
- Esumi, K., Wakabayashi, M., & Torigoe, K. (1996). Preparation of colloidal silver-palladium alloys by UV-irradiation in mixtures of acetone and 2propanol. Colloid and Surface A-Physicochemical and Engineering Aspects, 109, 55–62.
- Finotelli, P. V., Morales, M. A., Rocha-Leao, M. H., Baggio-Saitovitch, E. M., & Rossi, A. M. (2004). Magnetic studies of iron (III) nanoparticles in alginate polymer for drug delivery applications. *Materials Science and Engineering C*, 24, 625–629.
- Gombotz, W. R., & Wee, S. F. (1998). Protein release from alginate matrices. Advanced Drug Delivery, 31, 267–285 (review).
- Hebeish, A. A., El-Rafie, M. H., Abdel-Mohdy, F. A., Abdel-Halim, E. S., & Emam, H. E. (2010). Carboxymethyl cellulose for green synthesis and stabilization of silver nanoparticles. *Carbohydrate Polymers*, 82, 933–941.
- Hirai, H. (1979). Formation and catalytic functionality of synthetic polymer–noble metal colloid. *Journal of Macromolecular Science-Chemistry*, A13, 633–649.

- Hirai, H., Nakao, Y., & Toshima, N. (1979). Preparation of colloidal transition metals in polymers by reduction with alcohols or ethers. *Journal of Macromolecular Science-Chemistry*, A13, 727–750.
- Hossam, E. E., Avinash, P. M., Barbora, S., Heinz, D., Bernhard, R., Alexandra, P., et al. (2013). Treatments to impart antimicrobial activity to clothing and household cellulosic-textiles, why "Nano"-silver? *Journal of Cleaner Production*, 39, 17–23.
- Huang, H. H., Ni, X. P., Loy, G. L., Chew, C. H., Tan, K. L., & Loh, F. C. (1996). Photochemical formation of silver nanoparticles in poly(N-vinylpyrrolidone). *Langmuir*, 12, 909–912.
- Huang, L., Liao, W., Ling, H., & Wen, T. (2009). Simultaneous synthesis of polyaniline nanofibers and metal (Ag and Pt) nanoparticles. *Materials Chemistry and Physics*, 116, 474–478.
- Kattumuri, V., Katti, K., Bhaskaran, S., Boote, E. J., Casteel, S. W., & Fent, G. M. (2007). Gum Arabic as a phytochemical construct for the stabilization of gold nanoparticles: In vivo pharmacokinetics and X-ray-contrast-imaging studies. Small, 3, 333–341.
- Khairou, K. S., Al-Gethami, W. M., & Hassan, R. M. (2002). Kinetics and mechanism of sol-gel transformation between sodium alginate polyelectrolyte and some heavy divalent metal ions with formation of capillary structure polymembranes ionotropic gels. *Journal of Membrane and Science*, 209, 445-456
- Kim, D., Park, S., Lee, J. H., Jeong, Y. Y., & Jon, S. (2007). Antibiofouling polymer-coated gold nanoparticles as a contrast agent for in vivo X-ray computed tomography imaging. *Journal of the American Chemical Society*, 129, 7661–7665
- Kiwi, J., & Graetzel, M. (1979). Projection, size factors, and reaction dynamics of colloidal redox catalysts mediating light induced hydrogen evolution from water. Journal of the American Chemical Society, 101, 7214–7217.
- Kuo, P. L., Chen, C. C., & Jao, M. W. (2005). Effects of polymer micelles of alkylated polyethyleneimines on generation of gold nanoparticles. *Journal of Physical Chemistry B*, 109, 9445–9450.
- Leung, T., Wong, C., & Xie, Y. (2010). Green synthesis of silver nanoparticles using biopolymers, carboxymethylated-curdlan and fucoidan. *Materials Chemistry and Physics*, 121, 402–405.
- Liu, Y., Chen, S., Zhong, L., & Wu, G. (2009). Preparation of high-stable silver nanoparticle dispersion by using sodium alginate as a stabilizer under gamma radiation. *Radiation Physics and Chemistry*, 78, 251–255.
- Luo, C., Zhang, Y., Zeng, X., Zeng, Y., & Wang, Y. (2005). The role of poly (ethylene glycol) in the formation of silver nanoparticles. *Journal of Colloid and Interface Science*, 288, 444–448.
- Marijnissen, W., van Osch, G., Aigner, J., van der Veen, S., Hollander, A., Verwoerd-Verhoef, H., et al. (2002). Alginate as a chondrocyte-deliver. *Biomaterials*, 23, 1511–1517
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Analytical Chemistry, 31(3), 426–428.
- Mohan, Y., Lee, K., Premkumar, T., & Geckeler, K. (2007). Hydrogel networks as nanoreactors: A novel approach to silver nanoparticles for antibacterial applications. *Polymer* 48, 158–164
- Mulvaney, P. (1996). Surface plasmon spectroscopy of nanosized metal particles. Langmuir. 12, 788–800.
- Nadezhda, V. I., Natalya, N. T., Lydmila, A. E., & Vasilyi, A. B. (2012). The study of the reaction of pectin-Ag (0) nanocomposites formation. *International Journal of Carbohydrate Chemistry*, 2012, 9 p.
- Nesterova, M. V., Walton, S. A., & Webb, J. (2000). Nanoscale iron (III) oxyhydroxy aggregates formed in the presence of functional water-soluble polymers: Models for iron (III) biomineralisation processes. *Journal of Inorganic Biochemistry*, 79, 109–118.
- Pal, A., Shah, S., & Devi, S. (2009). Microwave-assisted synthesis of silver nanoparticles using ethanol as a reducing agent. *Materials Chemistry and Physics*, 114, 530–532.
- Rastogi, L., & Arunachalam, J. (2011). Sunlight based irradiation strategy for rapid green synthesis of highly stable silver nanoparticles using aqueous garlic (Allium sativum) extract and their antibacterial potential. Materials Chemistry and Physics. 129. 558–563.
- Robitaille, R., Pariseau, J. F., Leblond, F. A., Lamoureux, M., Lepage, Y., & Halle, J. P. (1999). Studies on small (<350 μm) alginate-poly-I-lysine microcapsules. III. Biocompatibility of smaller versus standard microcapsules. *Journal of Biomedical Materials and Research*, 44, 116–120.
- Roe, D., Karandikar, B., Bonn, N., Gibbins, B., & Roullet, J. (2008). Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. *Journal of Antimi*crobial and Chemotherapy, 61, 869–876.
- Saha, S., Pal, A., Kundu, S., Basu, S., & Pal, T. (2010). Photochemical green synthesis of calcium-alginate-stabilized ag and au nanoparticles and their catalytic application to 4-nitrophenol reduction. *Langmuir*, 26, 2885–2893.
- Saha, S., Pal, A., Pande, S., Sarkar, S., Panigrahi, S., & Pal, T. (2009). Room temperature ferromagnetic Ni nanocrystals: An efficient transition metal platform for manifestation of surface-enhanced raman scattering. *Journal of Physical Chemistry C*, 113, 7553–7560.
- Sreeram, K. J., Shrivastava, Y. H., & Nair, B. U. (2004). Studies on the nature of interaction of iron (III) with alginates. BBA-General Subjects, 1670, 121–125.
- Sumner, J. B. (1921). Dinitrosalicylic acid: A reagent for the estimation of sugar in normal and diabetic urine. *Journal of Biology and Chemistry*, 47, 5–9.
- Sun, X., Dong, S., & Wang, E. (2004). One-step synthesis and characterization of polyelectrolyte-protected gold nanoparticles through a thermal process. *Poly*mer, 45, 2181–2184.

- Sun, X., Dong, S., & Wang, E. (2005). One-step preparation of highly concentrated well-stable gold colloids by direct mix of polyelectrolyte and HAuCl<sub>4</sub> aqueous solutions at room temperature. *Journal of Colloid and Interface Science*, 288, 301–303
- Sun, Y., & Xia, Y. (2002). Shape-controlled synthesis of gold and silver nanoparticles. Science, 298, 2176–2179.
- Tankhiwale, R., & Bajpai, S. (2009). Graft copolymerization onto cellulose-based filter paper and its further development as silver nanoparticles loaded antibacterial food-packaging material. *Journal of Colloids Surface B*, 69, 164–168.
- Toshima, N., Harada, M., Yamazaki, Y., & Asakura, K. (1992). Catalytic activity and structural analysis of polymer-protected gold-palladium bimetallic clusters prepared by the simultaneous reduction of hydrogen tetrachloroaurate and palladium dichloride. *Journal of Physical Chemistry*, 96, 9927–9933.
- Travan, A., Pelillo, C., Donati, I., Marsich, E., Benincasa, M., Scarpa, T., et al. (2005). Infection and the chronic wound: A focus on silver. *Advances in Skin and Wound Care*, 18, 2–12.
- Yamamoto, M., & Nakamoto, M. (2003). Novel preparation of monodispersed silver nanoparticles via amine adducts derived from insoluble silver myristate in tertiary alkylamine. *Journal of Materials Chemistry*, 13, 2064– 2065.
- Yoksan, R., & Chirachanchai, S. (2009). Silver nanoparticles dispersing in chitosan solution: Preparation by Gamma ray irradiation and their antimicrobial activities. *Materials Chemistry and Physics*, 115, 296–302.
- Yonezawa, T., & Toshima, N. (1995). Mechanistic consideration of formation of polymer-protected nanoscopic bimetallic clusters. *Journal of the Chemical Society, Faraday Transactions*, 91, 4111–41119.